SUPPLEMENTAL MATERIAL

Cholic Acid-Peptide Conjugates (CAPs) as Potent Antimicrobials against Interkingdom Polymicrobial Biofilms

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Table S1. Table showing antibacterial and antifungal activities of CAPs (**1-20**) against different bacterial and fungal strains along with cytotoxicity activities of the amphiphiles against human lung epithelial cells. All values mentioned are in μg/mL.

		MIC_{99} (μg/mL)[a] (Gram-positive bacteria)			MIC_{80} (μg/mL)[b] (*Candida* strains)			IC_{50} (μg/mL)[c]
CAPs		*S. aureus*	*B. subtilis*	*S. pneumoniae*	*C. albicans*	*C. tropicalis*	*C. kefyr*	A549
CAP **1**	CA-G_3	121.6	60.8	30.4	3.8	7.6	3.8	>95.03
CAP **2**	CA-A_3	127.0	63.5	15.9	4.0	4.0	2.0	75.81
CAP **3**	CA-V_3	8.6	8.6	8.6	4.3	4.3	2.1	60.74
CAP **4**	CA-I_3	8.9	8.9	8.9	4.5	8.9	2.2	8.59
CAP **5**	CA-L_3	35.8	35.8	8.9	8.9	4.5	4.5	11.05
CAP **6**	CA-P_3	136.6	136.6	17.1	8.5	8.5	2.1	>106.75
CAP **7**	CA-M_3	18.8	18.8	75.1	4.7	9.4	4.7	68.79
CAP **8**	CA-F_3	>312.5	>312.5	78.1	9.8	9.8	4.9	4.87
CAP **9**	CA-Y_3	>324.8	>324.8	>324.8	20.3	>324.8	5.1	>116.87
CAP **10**	CA-W_3	291.3	291.3	291.3	291.3	291.3	145.6	>113.78
CAP **11**	CA-S_3	133.2	133.2	66.6	8.3	8.3	4.2	>104.04
CAP **12**	CA-T_3	277.1	277.1	69.3	17.3	8.7	8.7	>108.25
CAP **13**	CA-H_3	80.9	80.9	40.4	10.1	40.4	5.1	>126.45
CAP **14**	CA-D_3	>287.9	>287.9	>287.9	>287.9	>287.9	>287.9	>112.44
CAP **15**	CA-E_3	>298.6	>298.6	>298.6	>298.6	>298.6	>298.6	>116.65
CAP **16**	CA-R_3	40.8	>326.3	10.2	20.4	>326.3	10.2	>249.56
CAP **17**	CA-C_3	>278.7	>278.7	139.3	>278.7	>278.7	4.4	>217.73
CAP **18**	CA-K_3	>325.9	163.0	40.7	5.1	20.4	5.1	>127.38
CAP **19**	CA-N_3	>287.1	>287.1	>287.1	17.9	17.9	9.0	>112.15
CAP **20**	CA-Q_3	148.7	148.7	>297.3	37.2	37.2	37.2	>116.36

a: Minimum inhibitory concentration at which 99% bacterial killing was observed. b: Minimum inhibitory concentration at which 80% fungal killing was observed. c: Cytotoxic activity of CAPs against human lung epithelial (A549) cells as IC_{50} the concentrations at which 50% cell death was observed. d: not determined.

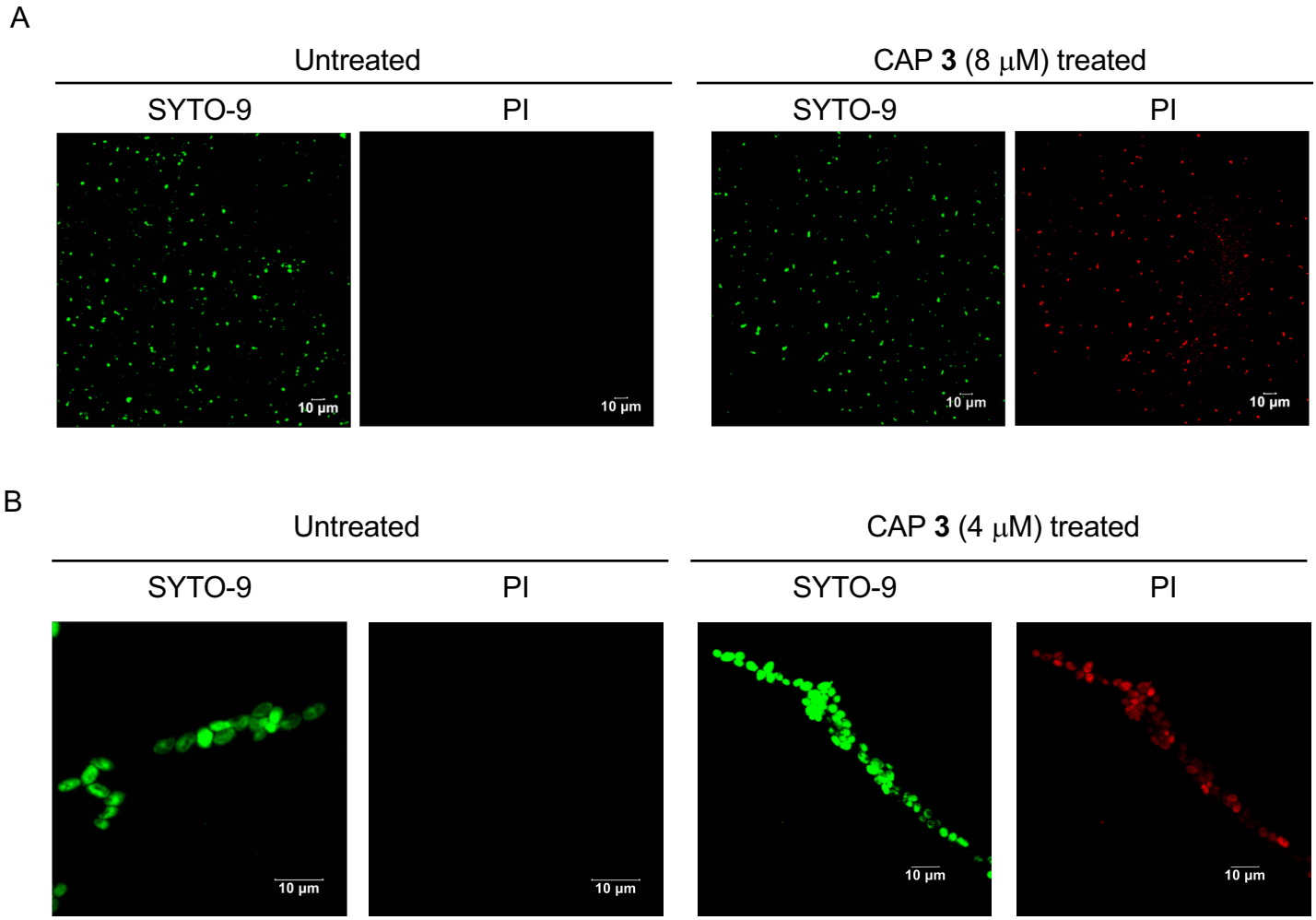

Figure S1. Fluorescence micrographs of *S. aureus* (A) and *C. albicans* (B) stained with SYTO9 and PI for live and dead cells after 6 h of CAP **3** treatment.

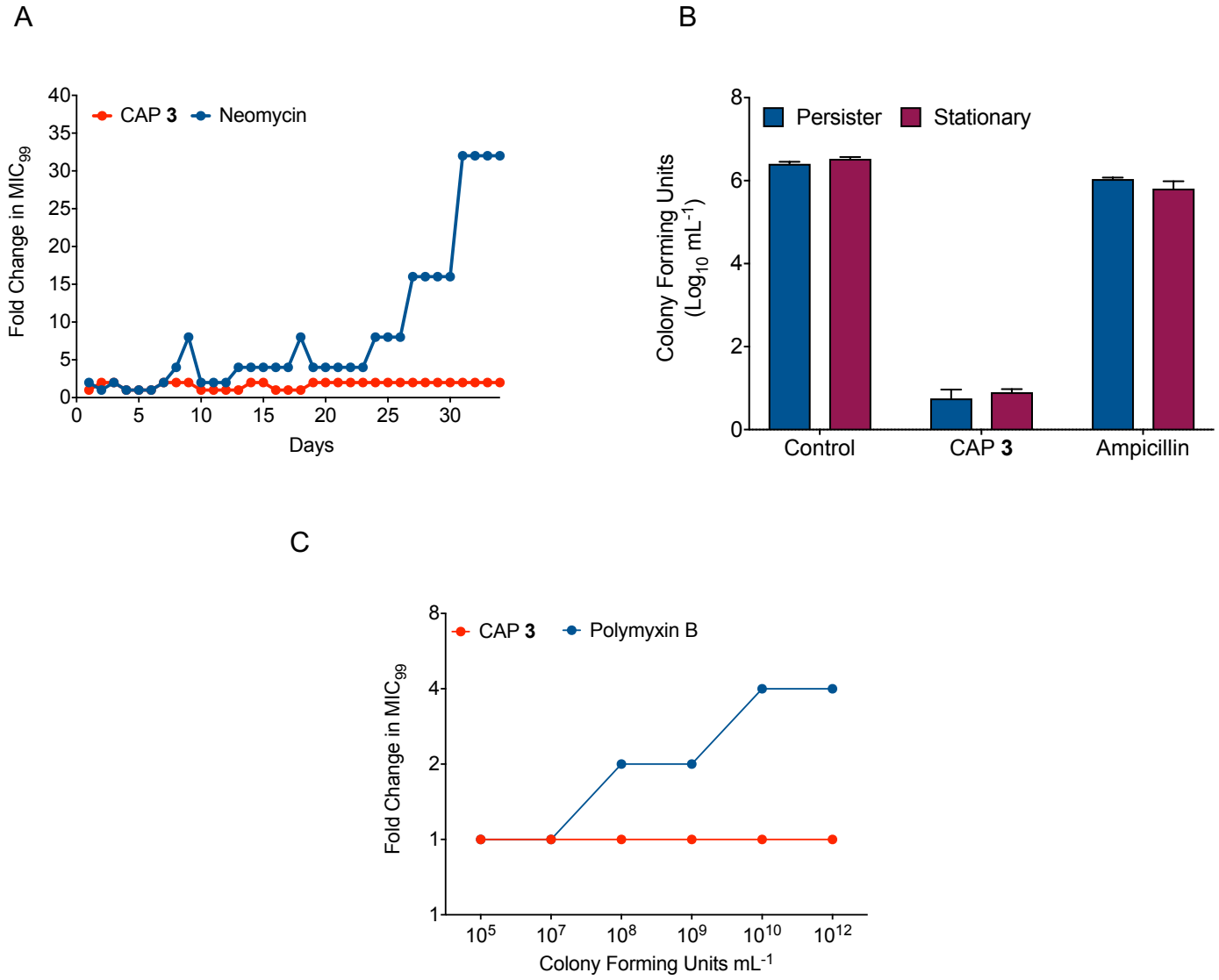

Figure S2. A) Change in MIC_{99} of CAP **3** against bacteria over multiple generations show the inability of the bacteria to generate many fold drug resistance against CAP **3** on sequential treatment as compared to neomycin for which bacteria develop resistance. B) Antibacterial activities of CAP **3** on stationary and persistent bacteria confirm the clearance of bacteria as compared to ampicillin. C) Mutant prevention assay witness the ability of CAP **3** to clear any persisters at higher CFU as compared to increase in MIC_{99} of polymyxin B at higher CFU.

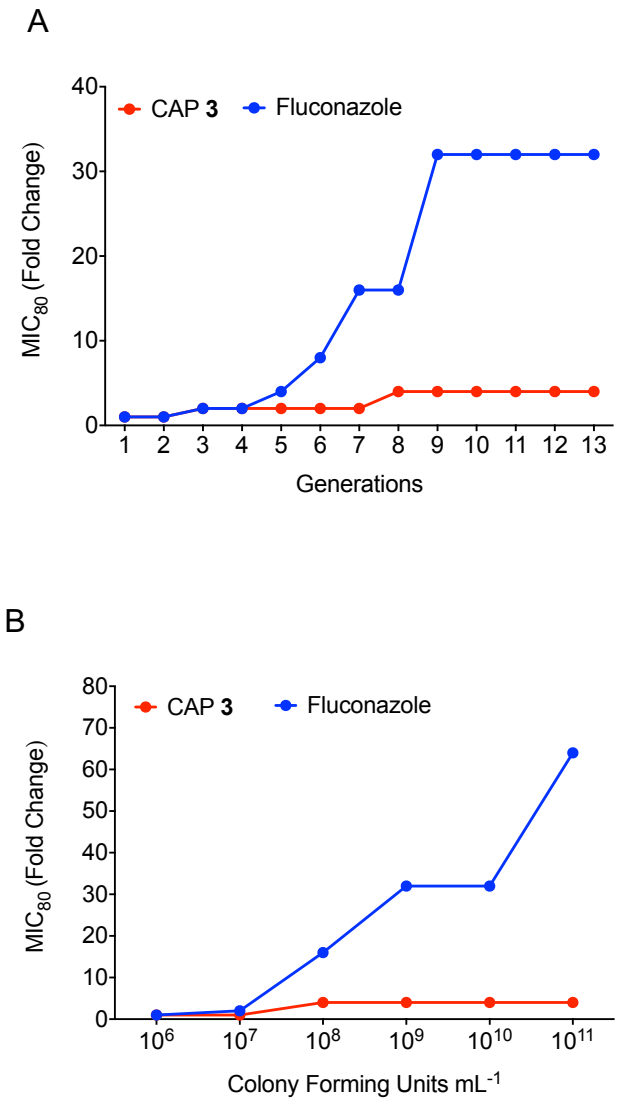

Figure S3. A) Change in MIC_{80} of CAP **3** against *C. albicans* confirm the inability of the *C. albicans* to generate drug resistance against CAP **3** whereas *C. albicans* develop many fold drug resistance to fluconazole. B) Mutant prevention assay witness the ability of CAP **3** to clear any *C. albicans* at higher CFU as compared to increase in MIC_{80} of fluconazole at higher CFU.

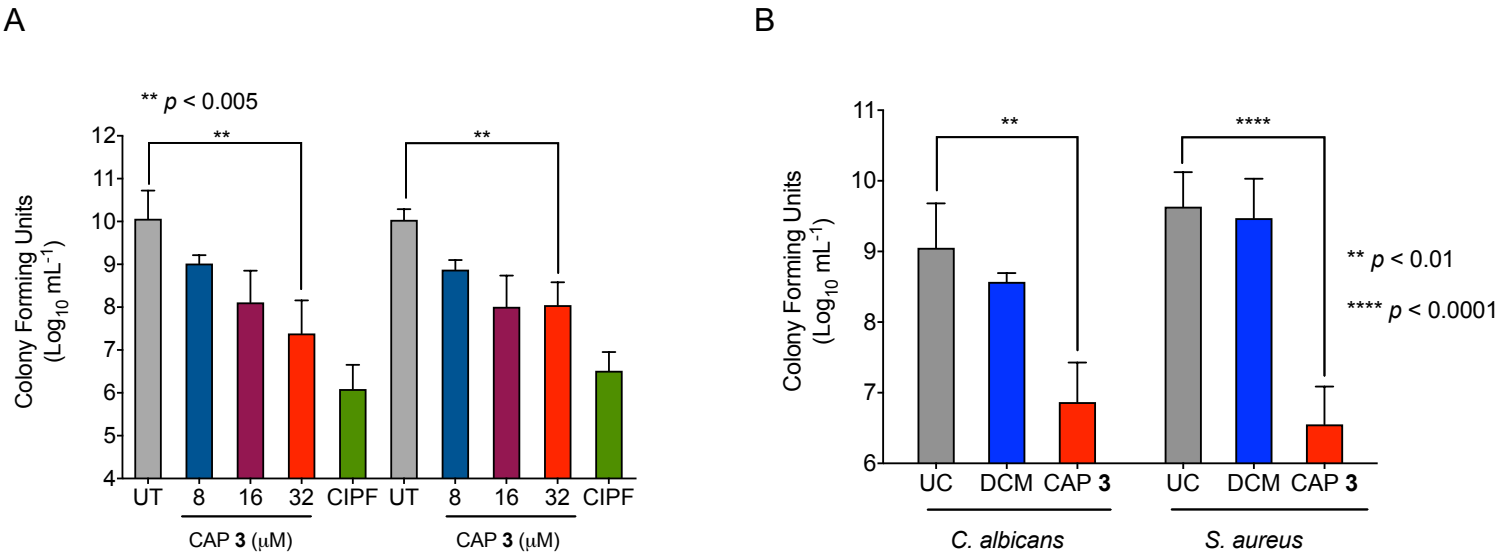

Figure S4. A) Quantification of *S. aureus* and *C. albicans* by colony forming units (CFU/mL) after treatment of pre-formed polymicrobial biofilms on catheters with different doses of CAP **3** (8, 16 and 32 μM) for 24 h confirm the ability of CAP **3** to degrade the existing pre-formed biofilms. Combination of ciprofloxacin (32 μM) and fluconazole (32 μM) (CIPF) was used as control. UT means untreated. B) Quantification of *S. aureus* and *C. albicans* on uncoated (UC), DCM- and CAP **3**-coated catheters (15 dips in 20 mg/mL of dichloromethane (DCM) solution of CAP **3**) by colony forming units witness the ability of the CAP **3** to prevent the formation of polymicrobial biofilm.